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Imaging Phenotype of Occupational Endotoxin-Related Lung Function Decline

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Short running title: CT phenotype of occupational endotoxin exposure

Contribution: PL, GW, and DC contributed to the conceptual design of this manuscript. PL, JH, FZ, JS, BZ, LS, and DC participated in the study implementation and data acquisition. DC is the parent study PI and established the cohort on which this study is based, and JH is the Shanghai local site investigator. PL, GW, DC participated in data analysis and interpretation of the results. PL participated in the writing of the manuscript with input from all authors. PL and DC had access to all the data and take responsibility for the integrity of the work.

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ABSTRACT

Background: Although occupational exposures contribute to a significant proportion of obstructive lung disease, the phenotype of obstructive lung disease associated with work-related organic dust exposure independent of smoking remains poorly defined.

Methods: The Shanghai Textile Worker Study is a longitudinal study of endotoxin-exposed cotton and endotoxin-unexposed silk workers initiated in 1981. Spirometry, occupational endotoxin exposure, and smoking habits were assessed at five year intervals. High-resolution computed tomography (CT) was performed in 464 retired workers in 2011, with quantitative lung densitometric and airway analysis.

Results: Significant differences in all CT measures were noted across exposure groups. Occupational endotoxin exposure was associated with a -1.3 % decrease in % emphysema ($LAA_{I\text{-}950}$), 3.3 hounsfield units increase in 15^{th} percentile density, 18.1 gram increase in lung mass, and a 2.3% increase in wall area %. Current but not former smoking was associated with a similar CT phenotype. Changes in $LAA_{I\text{-}950}$ were highly correlated with 15^{th} percentile density (correlation -1.0). Lung mass was the only measure associated with FEV_1 decline, with each 10 gram increase in lung mass associated with an additional loss of -6.1 mL of FEV_1 ($p=0.001$) between 1981 and 2011.

Conclusions: There are many similarities between the effect of occupational endotoxin exposure and tobacco smoke exposure on lung parenchyma and airway remodeling. The effects of occupational endotoxin exposure appear to persist even after exposure cessation. $LAA_{I\text{-}950}$ may not be a reliable indicator of emphysema in subjects without spirometric impairment. Lung mass is a CT-based biomarker of accelerated lung function decline.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is the fifth leading cause of death in developed countries (Collaborators 2015). Recent population based studies reported a population attributable risk of occupational exposures to COPD of 24% overall and 51% among non-smokers (Mehta et al. 2012). Much of the research characterizing the phenotype and severity of COPD has been performed in smokers (Marchetti et al. 2014; Paulin et al. 2015), where an additional limitation is that all occupational exposures are grouped together under the category of "vapors, gases, dusts, and fumes". It is well established that different occupational exposures lead to different health effects (Rom and Markowitz 2006), with only some associated with a risk of COPD (Matheson et al. 2005).

Exposure to biological dust is associated with an increased risk of COPD (Matheson et al. 2005). Furthermore, exposure to endotoxin in organic dust is common, with high-level exposures measured in both work environments (farms, cotton processing, and animal care facilities (Liebers et al. 2008)) and non-work environments (schools (Jacobs et al. 2013) and in homes burning biomass fuel (Semple et al. 2010)), highlighting the public health relevance of this exposure. Repeated inhalation of endotoxin at levels approximating occupational settings has led to the development of both emphysema (Brass et al. 2008) and airways disease (Brass et al. 2003) in murine models. Therefore, the study of lung disease in the setting of occupational endotoxin exposure represents an opportunity to further characterize the phenotype of COPD associated with an important environmental exposure.

The Shanghai Textile Worker Study is an occupational cohort that has been followed longitudinally since 1981. Unique to this cohort is exposure characterization over the entire

working lifetime of the participants, large proportion of non-smokers, and little loss to follow-up, with 74% of the original participants still alive participating in the 30 year survey. For decades, controversy has existed surrounding whether emphysema or airways disease forms the basis for the chronic airflow obstruction noted in the setting of endotoxin-containing cotton dust exposure. Prior autopsy studies have demonstrated both, although these studies were unable to distinguish between disease due to concurrent smoking vs. disease due to work exposure (Edwards et al. 1975; Pratt et al. 1980; Schachter et al. 1980). In this study, our primary aim was to identify the relative contribution of smoking vs. occupational endotoxin exposure on parenchymal and airway remodeling as defined by quantitative computed tomography (CT). Our secondary aim was to identify imaging biomarkers associated with lung function decline.

METHODS

Study population and study design

The Shanghai Textile Worker Study was designed based on the observation that cotton dust contains high levels of endotoxin, whereas silk dust contains undetectable levels of endotoxin, representing a natural experiment to evaluate the effect of occupational endotoxin exposure on lung disease. In 1981, 919 Han Chinese workers from two cotton and one silk textile mill in the same industrial sector in Shanghai, China were recruited (study schema in **Figure 1**); 90% of those eligible were enrolled (Christiani et al. 1994). The main inclusion criterion was at least two years of work in the identified mills in order to ensure a stable study population; subjects with a history of prior respiratory disease were excluded. Cotton and silk workers were comparable with respect to income, place of residence, and other socio-economic factors due to the hiring practices of the Shanghai Textile Bureau. Surveys were performed in 1981, 1986, 1992, 1996,

2001, 2006, and 2011, with eligibility for retesting based on presence in the baseline 1981 survey. Pre-bronchodilator spirometry performed according to American Thoracic Society guidelines, physical exam, modified American Thoracic Society symptom, work history, and smoking questionnaires, and exposure assessment (in the period prior to worker retirement) was performed at each survey.

In 2011, 464 subjects consented to volumetric chest CT scans performed at full inspiration and expiration using a single Siemens Emotion-16 CT Scanner. Images were obtained at 0.75mm slice thickness and reconstructed using a B65s reconstruction kernel. Airway Inspector software (San Jose Estepar et al. 2008) was used to obtain measures of both lung attenuation and airway morphology. In addition, all scans were interpreted based on a consensus read of two radiologists reading simultaneously onto a standardized CT assessment score sheet to identify the presence of emphysema.

Informed consent was obtained from all subjects and the study was approved by the Institutional Review Boards at the Harvard School of Public Health and the Shanghai Putuo District People's Hospital.

Exposure assessment

Exposure assessment was performed as previously described (Kennedy et al. 1987; Olenchock et al. 1990). Between 1981 and 2001 (after which most workers retired), multiple area samples were collected from each of the 6 different work areas in the two cotton mills and one silk mill using vertical elutriators to collect respirable fractions of dust, with sampling times ranging from 3 to 7 hours. All collected dust samples were weighed to estimate exposure to respirable dust,

whereas dust from all cotton mills and a limited number of full shift samples from silk mills during that period were sent to a single lab at the National Institute of Occupational Safety and Health to quantify endotoxin content using a Limulus amebocyte lysate gel test (Pyrostat-50). Values for each filter were summed and converted from ng/ml to $\mu g/m^3$ based on sampling time and air flow rates of each sampler. The lower limit of detection for endotoxin by this method was 0.001 EU/m^3. Exposure measurements collected in the first survey were used to estimate pre-1981 exposure by area, and area exposure measurements in 2001 were used to approximate post-2001 area exposures. 6 full-shift samples in the silk mills confirmed near-undetectable levels of endotoxin (0.001 EU/m^3) in vertical elutriator samples; thus silk workers were considered exposed to negligible amounts of endotoxin at work. Individual endotoxin exposure was calculated using geometric means of endotoxin measured in each work area multiplied by years of work in each work area, resulting in a lifetime cumulative index of occupational exposure measured in endotoxin units/$meters^3$-years (EU/m^3-yrs), with an interpretation analogous to that of pack-years for smoking. At each survey, a detailed work history was obtained to identify the date of textile work cessation as well as job descriptions post retirement in order to account for occupational endotoxin exposure after officially retiring from the mills.

Outcome measures

The primary outcome measures were CT measures of parenchymal remodeling and airway morphology. To evaluate parenchymal remodeling, we evaluated the following measures: 1) percent emphysema defined by the percentage of low attenuation area of lung less than -950 Hounsfield Units (HU) at full inspiration (LAA_{-950}); 2) 15^{th} percentile density which is the HU threshold demarcating the lowest 15^{th} percent of lung attenuation (PD15) (Parr et al. 2008); and

3) lung mass (Henne et al. 2012), based on studies suggesting that emphysematous destruction of the lung parenchyma may be associated with increased lung mass due to inflammation and remodeling(Guenard et al. 1992).

Measures of central airway morphology to identify wall area % (WA %) was obtained in all subjects from the apical segment of the right upper lobe, a third generation segmental airway. Airway segmentation was performed using phase congruency as described previously (Estepar et al. 2006).

This investigation utilized a single CT scanner that was calibrated to ensure accuracy. The protocol consisted of daily air calibrations as well as periodic water calibrations per the vendor's recommendations. Air and water have known attenuation values of -1000 HU and 0 HU respectively, with attenuation values for emphysema (Gevenois et al. 1995), normal lung (Coxson et al. 1999), and interstitial abnormalities (Lederer et al. 2009) falling within this range. Validation of the extremes of this range allows accurate densitometric discrimination of processes affecting the lung tissue."

The secondary outcome measure was change in FEV_1 between 1981 and 2011, calculated as the difference in FEV_1 measured at these two time points.

Statistical Analysis

In the primary outcome analysis, linear regression was used to determine the association between exposure and each CT outcome measure. Occupational endotoxin exposure was modeled in one of two ways; either using cotton vs. silk work as a binary variable, or as log-transformed

cumulative occupational endotoxin exposure. Smoking exposure was modeled using smoking status (defined as never, ever, or former), and cumulative pack-years smoked. All multivariable analyses were adjusted for age, gender, height, body mass index, duration of work cessation years, and inflation level. For inspiratory measures, inflation level was calculated using CT measured total volume divided by predicted total lung capacity (Grydeland et al. 2010). Sensitivity analyses were performed where the analysis was restricted to cotton workers (given that only a limited number of full shift measures for endotoxin was performed in the silk workers), non-smokers, and cotton non-smokers. Additional sensitivity analyses were tested with models incorporating interaction terms between occupational and smoking exposure.

In the secondary outcome measure, additional covariates included were CT measures of remodeling, in order to identify predictors of lung function decline.

All analyses were performed in R version 3.1. Two-sided p-values of < 0.05 were considered significant.

RESULTS

Characteristics of the 464 subjects are as described in **Table 1**. Most (70.2%) were lifetime nonsmokers and 52.6% were cotton workers. All subjects had retired from active textile work, with the average duration of retirement being 17.7 ± 4.6 years. 168 (36.2%) were male, and the average age was 63.6 ± 8.7 years old. Notably, 93% of all smokers were male whereas 89% of all nonsmokers were female. Unlike smoking exposure, occupational exposure was not stratified by gender; 35% of silk workers and 37.2% of cotton workers were male. The average % predicted FEV_1 was 109.7 ± 17.8%, with 7 of the non-smokers and 16 of the smokers having

FEV_1/FVC less than the lower limit of normal. Annual decline in FEV_1 was -15.1, -18.3, -28.4, and -31.9 mL/year for non-smoking silk, non-smoking cotton, smoking silk, and smoking cotton workers respectively (p=0.008 for linear trend).

Quantitative CT characteristics of the study population are as depicted in **Table 2**. There were significant differences in CT measures across all exposure groups. In these unadjusted analyses, cotton work was associated with lower % emphysema, higher 15th percentile density, higher lung mass, higher wall area %, whereas smoking was associated with higher % emphysema, lower 15th percentile density, higher lung mass, and lower wall area %. It must be emphasized that most smokers were male and most non-smokers were female; large gender differences in quantitative CT measures have been previously described with men having higher % emphysema than women (Grydeland et al. 2009; Hoffman et al. 2014).

The associations between occupational or smoking exposure and quantitative CT measures in multivariate models are shown in **Table 3**. In the overall cohort, cotton work was associated with significant decreases in % emphysema, increases in 15th percentile density, lung mass, and wall area %. When occupational exposure was modeled as cumulative endotoxin exposure, a dose-response relationship was seen. Increased endotoxin exposure was associated with decreased % emphysema, increased 15th percentile density, increased lung mass, and increased wall area %.

When the analysis was restricted to cotton workers (n = 244), an association between occupational endotoxin exposure and lung mass was still detected, with a trend towards significance in the association between occupational exposure and % emphysema and 15th percentile density. When the analysis was restricted to cotton nonsmokers (n = 164), significant associations between occupational endotoxin exposure and decreased % emphysema, increased

15th percentile density, and increased lung mass was detected. When the analysis was restricted to nonsmokers (n = 322), very similar effect estimates were seen. None of the interaction terms between occupational and smoking exposure were significant for any of the outcomes.

In the overall cohort, the adjusted effect of smoking on quantitative CT measures was very similar in direction to that of occupational endotoxin exposure. Current as compared to never smokers had lower % emphysema, higher 15th percentile density, higher lung mass, and non-significant increases in wall area %. However, this association was not seen when comparing former vs. never smokers or when evaluating pack-years.

To better understand the unexpected association between occupational or smoking exposures and decreased % emphysema as measured by LAA_{I-950}, additional analyses were performed. Measures of LAA_{I-950} were found to be highly correlated with 15th percentile density (Pearson correlation -0.87, Spearman correlation -1.00), with lower measures of % emphysema associated with higher 15th percentile density in all exposure groups (**Figure 2**). Additionally, all CT scans were reviewed by two radiologists for the presence or absence of emphysema in order to compare the distribution of LAA_{I-950} in those with vs. without emphysema. Average LAA_{I-950} was 13.9% vs. 12.7% in those with vs. without emphysema (p=0.06). Notably, LAA_{I-950} ranged from 0.7 to 30.6% vs. 0.8 to 31.0% in those with vs. without emphysema, indicating significant overlap.

In multivariable analyses, lung mass was the only CT measure significantly associated with lung function decline from 1981 to 2011 (**Table 4**). Each 10 gram increase in lung mass is associated with an additional loss of -6.l mL of FEV_1 (p=0.001) over this 30 year period.

DISCUSSION

This is the first comprehensive occupational study evaluating the relative contributions of lifetime occupational endotoxin and smoking exposure to the phenotype of lung function decline as defined by quantitative lung CT. In our cohort, we found that workplace exposures lead to a phenotype of lung disease very similar to smoking exposure, with increased lung density and increased lung mass. Occupational endotoxin exposure was also associated with increased airway wall thickening in the overall cohort. $LAA_{I\text{-}950}$ did not appear to be a reliable measure of emphysema when compared to radiologist review in our cohort, and may reflect changes in overall lung density rather than the presence of emphysema. Lung mass was the only CT biomarker associated with longitudinal FEV_1 decline.

Our study is the only available study that quantifies the degree to which lifetime occupational endotoxin exposure in cotton textile workers contributes to the phenotype of lung disease as defined by quantitative lung CT. There is only one other study investigating the CT phenotype of lung disease associated with occupational exposures (Marchetti et al. 2014); this was based on the COPDGene study, where participants were all current or former smokers and 86% met spirometric criteria for COPD. Self-reported ever exposure to dust or fumes was associated with increased % emphysema, in contrast to our findings. However, only 10.8% of our cohort met spirometric criteria for COPD due to the healthy worker survivor effect common in many occupational studies.

An apparent "paradoxical" increase in CT measures of % emphysema has been noted when smokers quit (Shaker et al. 2011). In a study of smokers with COPD followed with annual CT scans, both quitting smoking and budesonide use was associated with an increase in %

emphysema and decrease in PD15. These activities are presumably anti-inflammatory and should not worsen emphysema. % emphysema has an almost perfect inverse correlation with PD15, and the decrease in % emphysema associated with both smoking and occupational exposures in our study likely reflects increased lung density due to inflammation, rather than an actual decrease in emphysema associated with these noxious environmental exposures. Another population-based study found lower % emphysema (defined by $LAA_{I\text{-}950}$) in current compared to former smokers, although the authors speculate that this is due to a healthy smoker survivor effect (Grydeland et al. 2009). The use of $LAA_{I\text{-}950}$ to quantify the extent of emphysema was originally validated in cohorts of subjects with spirometric COPD; it is unclear, as we have found, whether this sensitive measure is well correlated with the extent of emphysema in healthier subjects as seen in population-based or occupational cohorts.

We found many phenotypic similarities between the effect of active smoking and occupational endotoxin exposure on parenchyma and airway changes. What is interesting is that while only active (and not former) smoking was associated with changes in lung density, prior occupational endotoxin exposure is associated with persistent changes in lung density and airway wall thickening. At the time imaging was performed, the average duration of retirement was 17.7 years in this cohort. The effects of occupational endotoxin exposure on lung morphology appears to persist even after exposure cessation. We have previously demonstrated that in this cohort, prior occupational endotoxin exposure is associated with a dose-related impairment in lung function recovery even after exposure cessation (Lai et al. 2015); this suggests that these persistent CT changes have functional significance.

The mechanism of these persistent CT changes despite exposure cessation is not clear as few animal and human studies have evaluated chronic rather than acute endotoxin exposure. We do

not think that subclinical interstitial lung disease due to occupational endotoxin exposure can explain the persistent increase in lung density and lung mass noted as 32 (6.9%) of silk workers and 31 (6.7%) of cotton workers had interstitial lung abnormalities based on a validated radiologist sequential reading method (Washko et al. 2010). A prior murine model of chronic endotoxin exposure demonstrated persistent lung neutrophilic inflammation that is correlated with an expansion of lung inflammatory dendritic cells (Lai et al. 2012), suggesting that if these persistent changes in lung density are due to persistent inflammation despite exposure cessation, then a plausible mechanism to explain these findings does exist.

We identified lung mass as the only CT-based biomarker we assessed for FEV_1 decline. While other studies have supported the association between accelerated FEV_1 decline and emphysema extent as measured by $LAA_{I\text{-}950}$ (Vestbo et al. 2011) or radiologist assessment (Nishimura et al. 2012), these studies were again performed in subjects with a spirometric diagnosis of COPD and may not apply to population-based or occupational cohorts without evidence of lung function impairment. Adjusting for age, gender, and anthropometric measures, each 10 gram increase in lung mass was associated with an additional 6.1 mL loss in FEV_1 over the study period. Lung mass as measured by CT has been validated against *ex vivo* measurements (Henne et al. 2012), and studies have shown the unexpected finding that patients with emphysema appear to have heavier rather than lighter lungs (Guenard et al. 1992). *Ex vivo* lungs from patients with emphysema have demonstrated that airspace enlargement is accompanied by an even greater increase in both elastin and collagen in the alveolar interstitium (Vlahovic et al. 1999). This finding provides a plausible mechanistic explanation for why lung mass was the most potent marker for disease activity as measured by lung function decline in our cohort; after adjusting for

age, gender, and anthropometric differences, it may be the optimal biomarker for inflammation or parenchymal remodeling.

Our study has several strengths. First, to our knowledge, this is the only study that is able to estimate measured workplace endotoxin exposures over the working lifetime of a cohort of cotton textile workers. Other occupational studies of lung disease rely on self-reported exposures, which are influenced by recall bias, or job exposure matrices, which cannot quantify actual exposures. Second, our cohort has a large proportion of non-smokers, while most other studies evaluating the contribution of occupational exposures to lung disease were performed largely in smokers. Third, follow-up in our cohort has spanned three decades, allowing us to obtain estimates of FEV_1 decline over this period of time. While cross-sectional measures of % predicted FEV_1 were within the normal range in this cohort, there were differences in FEV_1 decline when comparing exposure groups and highlights the importance of longitudinal studies when assessing the impact of exposure on respiratory outcomes. Our findings suggest that in the absence of longitudinal lung function data, cross-sectional CT measures of lung mass may serve as a biomarker for accelerated lung function decline.

Our study also has several limitations. First, smoking and gender are largely confounded, as most smokers were men, and most non-smokers were female. However, an analysis restricted to non-smokers and cotton nonsmokers showed very similar results as in our overall cohort, supporting our conclusions. Second, cotton dust may contain other microbial (Rylander et al. 1985) or bioactive (Buck et al. 1986) compounds for which endotoxin may serve as a proxy. We did not measure other bioactive agents in cotton dust beyond endotoxin although the association between endotoxin as the agent in cotton dust leading to health effects has been the most robustly supported in the literature (Lai and Christiani 2013). Third, because of the healthy worker

survivor effect seen in many occupational cohorts, only 10.8% of our cohort met spirometric criteria for COPD, and longitudinal FEV_1 changes were small compared to population controls. However, an important research initiative is the detection of subclinical disease using non-invasive imaging methods in order to identify individuals at a stage where interventions can prevent disease development or progression. Therefore, our finding of lung mass as an important biomarker for FEV_1 decline in both smoking and occupational organic dust exposure suggests that it can be more broadly applied to other COPD-related exposures.

CONCLUSIONS

In conclusion, in this workplace-based cohort of occupational organic dust and smoking exposure, we find that occupational endotoxin exposure is associated with persistent increases in lung density, lung mass, and airway thickening even after exposure cessation. CT measures of % emphysema using $LAA_{I\text{-}950}$ are likely more reflective of lung density rather than emphysema in populations without overt clinical disease, and should be interpreted with caution. Lung mass represents a potential CT-based biomarker for lung function decline, and should be further validated in future studies.

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Table 1. Baseline characteristics of study participants stratified by exposure level in 2011.

	Silk Non-smoker	Cotton Non-smoker	Silk Smoker	Cotton Smoker
Observations, n	158	164	62	80
Age, yrs	63.5 ± 8.8	63.8 ± 8.7	65.8 ± 9.9	63.8 ± 9.3
Male	17 (10.8%)	19 (11.6%)	60 (96.8%)	72 (90.0%)
Current smoking	0 (0%)	0 (0%)	43 (69.4%)	58 (72.5%)
Pack-years	0	0	28.4 ± 20.5	27.5 ± 18.6
Height, cm	157.9 ± 6	159.2 ± 6.5	167.2 ± 6.1	168.2 ± 7.2
Body Mass Index, cm/kg^2	23.8 ± 3.0	24.9 ± 3.6	24.0 ± 3.2	24.6 ± 3.2
Follow-up time, years	29.4 ± 0.1	29.6 ± 0.1	29.3 ± 0.0	29.6 ± 0.0
Work duration, years	25.4 ± 8.2	24.5 ± 7.3	28.5 ± 9.7	26.6 ± 7.9
Retirement duration, years	18.4 ± 4.1	17.4 ± 5.1	17.4 ± 4.3	16.9 ± 4.7
Cumulative endotoxin exposure, EU/m^3-years [a]	0	38,233.8 ± 31,962.6	0	61,123.6 ± 548,49.7
FEV_1, mL	2159.8 ± 440.4	2193.0 ± 491.5	2683.3 ± 678.0	2540.3 ± 811.7
FEV_1, % predicted	112.0 ± 16.8	111.4 ± 14.7	110.4 ± 21.3	100.4 ± 20.5
FEV_1/FVC	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
$FEV_1/FVC < 0.7$	15 (9.5%)	14 (8.5%)	19 (30.6%)	19 (23.8%)
$FEV_1/FVC < LLN$	4 (2.5%)	3 (1.8%)	9 (14.5%)	7 (8.8%)
Annual FEV_1 decline, mL/yr [b]	-15.1 ± 10.4	-18.3 ± 9.4	-28.4 ± 11.8	-31.9 ± 13.6
FEV_1 change since 1981, mL[c]	-442.0 ± 304.2	-542.3 ± 179.5	-832.8 ± 347.3	-941.9 ± 401.2
Any respiratory symptoms	38 (24.1%)	38 (23.2%)	22 (35.5%)	29 (36.2%)
Chronic bronchitis	6 (3.8%)	8 (4.9%)	9 (14.5%)	14 (17.5%)
Chronic cough	4 (2.5%)	3 (1.8%)	3 (4.8%)	3 (3.8%)
Dyspnea	35 (22.2%)	36 (22.0%)	14 (22.6%)	21 (26.2%)

[a] A limited number of full shift samples taken in silk mills were found to have endotoxin levels less than the lower limit of normal (0.001 EU/m^3) by the limulus amoebocyte lysate assay. Thus silk workers were considered exposed to negligible amounts of endotoxin at work.
[b] Calculated by taking difference in FEV_1 between 2011 and 1981 divided by elapsed time. Significant differences in annual FEV_1 decline noted between exposure groups, $p = 0.008$ for linear trend
[c] Significant differences in FEV_1 between 1981 and 2011, $p < 0.001$ for linear trend

Table 2. Quantitative Computed Tomography Characteristics of Study Population.

	Silk Non-smoker	**Cotton Non-smoker**	**Silk Smoker**	**Cotton Smoker**	p-value[a]
% emphysema (LAAI-950)	12.5 ± 6.2	11.9 ± 5.6	16.3 ± 6.2	14.0 ± 6.2	<0.001
15th percentile density (PD15)	-938.3 ± 26.5	-936.8 ± 27.0	-950.1 ± 22.6	-942.8 ± 25.7	0.004
Lung Mass, gm	616.5 ± 80.4	653.7 ± 91.4	775.7 ± 120.7	799.4 ± 123.5	<0.001
Wall area %	59.9 ± 6.4	62.7 ± 6.5	58.7 ± 6.2	60.0 ± 6.2	<0.001

[a] based on one-way ANOVA

Table 3. Multivariate mean differences in computed tomography measures of lung parenchyma and airway remodeling based on exposure.[a] Results based on all participants (n=464) and restricted to cotton workers (n = 244), non-smokers (n = 322), and cotton non-smokers (n = 164). Interaction terms between occupational exposure and smoking were non-significant.

	Group	% emphysema ($LAA_{I\text{-}950}$)	15th percentile density (PD15)	Lung Mass	Wall Area %
Cotton vs. silk	All	-1.26*** [-2.06, -0.46]	3.30** [0.12, 6.48]	18.10*** [4.52, 31.68]	2.32*** [1.17, 3.48]
Cumulative endotoxin, log EU/m^3	All	-0.05*** [-0.07, -0.02]	0.12** [0.01, 0.24]	0.67*** [0.20, 1.15]	0.08*** [0.04, 0.12]
Current vs. never smoker	All	-2.39** [-4.20, -0.58]	10.11*** [2.91, 17.30]	47.38*** [16.66, 78.10]	1.87 [-0.71, 4.46]
Former vs. never smoker	All	0.44 [-1.36, 2.25]	0.14 [-7.02, 7.30]	-0.56 [-31.13, 30.02]	0.27 [-2.32, 2.87]
Pack-years	All	0.01 [-0.03, 0.04]	-0.07 [-0.22, 0.08]	0.23 [-0.41, 0.87]	-0.03 [-0.08, 0.03]
Cumulative endotoxin, log EU/m3	Cotton	-0.30* [-0.65, 0.04]	1.33* [-0.06, 2.73]	6.77** [0.91, 12.63]	-0.36 [-0.88, 0.16]
Current vs. never smoker	Cotton	-1.37 [-3.78, 1.03]	8.89* [-0.84, 18.63]	33.61 [-7.37, 74.58]	-0.35 [-3.94, 3.24]
Former vs. never smoker	Cotton	0.08 [-2.32, 2.47]	2.45 [-7.24, 12.13]	4.19 [-36.57, 44.95]	0.15 [-3.45, 3.75]
Pack-years	Cotton	-0.001 [-0.06, 0.05]	-0.08 [-0.30, 0.14]	-0.07 [-1.00, 0.85]	0.03 [-0.05, 0.11]
Cotton vs. silk work	Non-smokers	-1.26*** [-2.06, -0.46]	3.30* [-0.57, 7.17]	21.18*** [5.63, 36.73]	2.94*** [1.56, 4.32]
Cumulative endotoxin, log EU/m3	Non-smokers	-0.05*** [-0.07, -0.02]	0.13* [-0.005, 0.27]	0.82*** [0.27, 1.37]	0.10*** [0.05, 0.15]
Cumulative endotoxin, log EU/m3	Cotton non-smokers	-0.75*** [-1.19, -0.31]	2.79*** [0.90, 4.69]	12.89*** [5.28, 20.49]	-0.003 [-0.68, 0.67]

* p<0.1; ** p<0.05; *** p<0.01

[a]All models adjusted for age, gender, height, body mass index, duration of work cessation years, and inflation or deflation level using CT measured volumes divided by predicted volumes.

Table 4. Quantitative CT measures associated with decline in FEV_1 between 1981 and 2011. Lung mass was the only CT measure associated with lung function decline. Each 10 gram increase in lung mass is associated with an additional loss of -6.l mL of FEV_1 over the 30 year study period.

	FEV_1 change (mL) [a]
% emphysema (LAA_{I-950}), %	1.26 [-3.53, 6.04]
15^{th} percentile density, Hounsfield units	-0.37 [-1.46, 0.72]
Lung Mass, grams	-0.61*** [-0.97, -0.24]
Wall Area %, %	0.48 [-4.14, 5.10]

* p<0.1; ** p<0.05; *** p<0.01
[a] FEV_1 change associated with 1 gram increase in lung mass
[b] All models adjusted for age, gender, height, body mass index, duration of work cessation years, occupational exposure (cotton vs. silk work), and smoking exposure (never, ever, former smoking as well as pack-years smoked).

FIGURE LEGENDS

Figure 1. Overview of Shanghai Textile Worker Study. Most of the workers retired between 1992 and 2001. Of the 587 subjects in the 2011 follow-up survey, 464 consented to and received high resolution computed tomography of the chest.

Figure 2. Correlation between CT measure of % emphysema (using cutoff of -950 Hounsfield Units, LAA $_{I\text{-}950}$) and 15th percentile density across all exposure subgroups. Correlation is high (Pearson correlation -0.87, Spearman correlation -1.00) and suggests that % emphysema measure may actually be reflecting changes in lung density, and not a true measure of emphysema.

Figure 1.

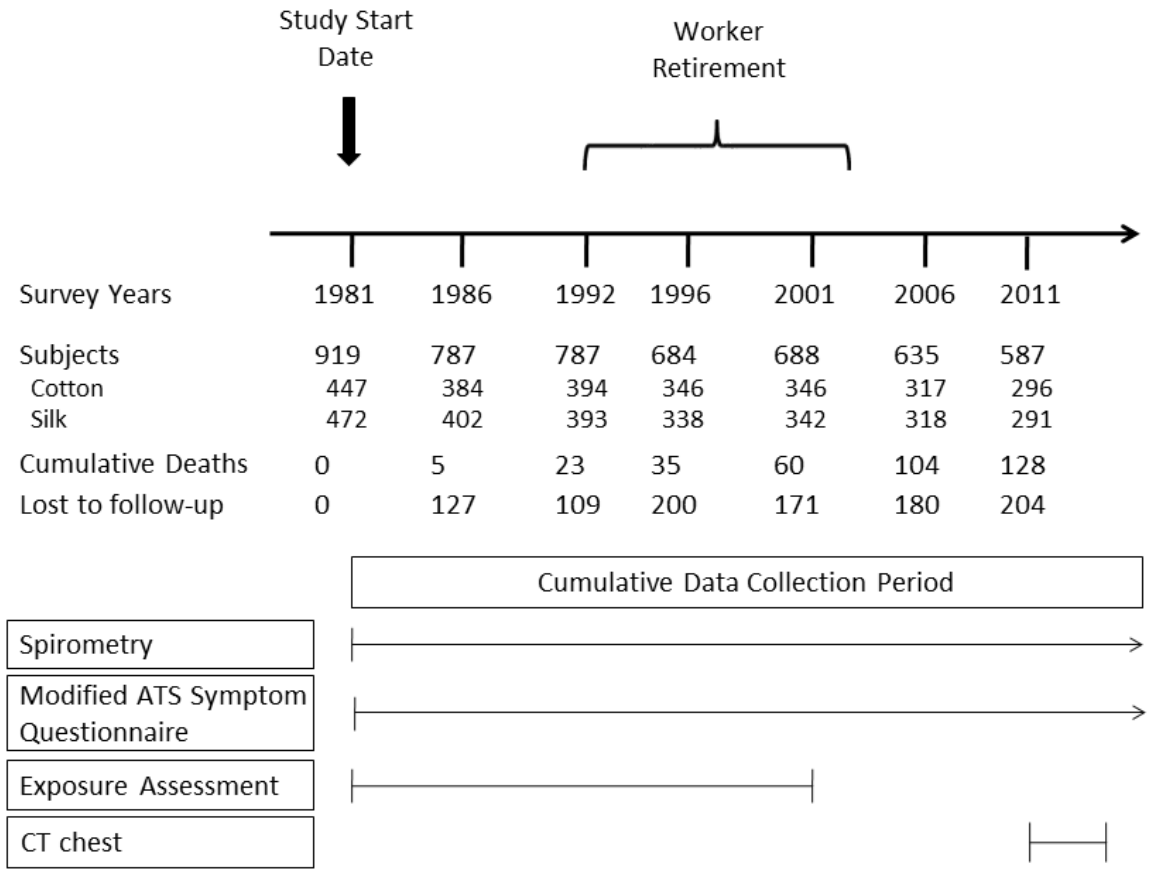
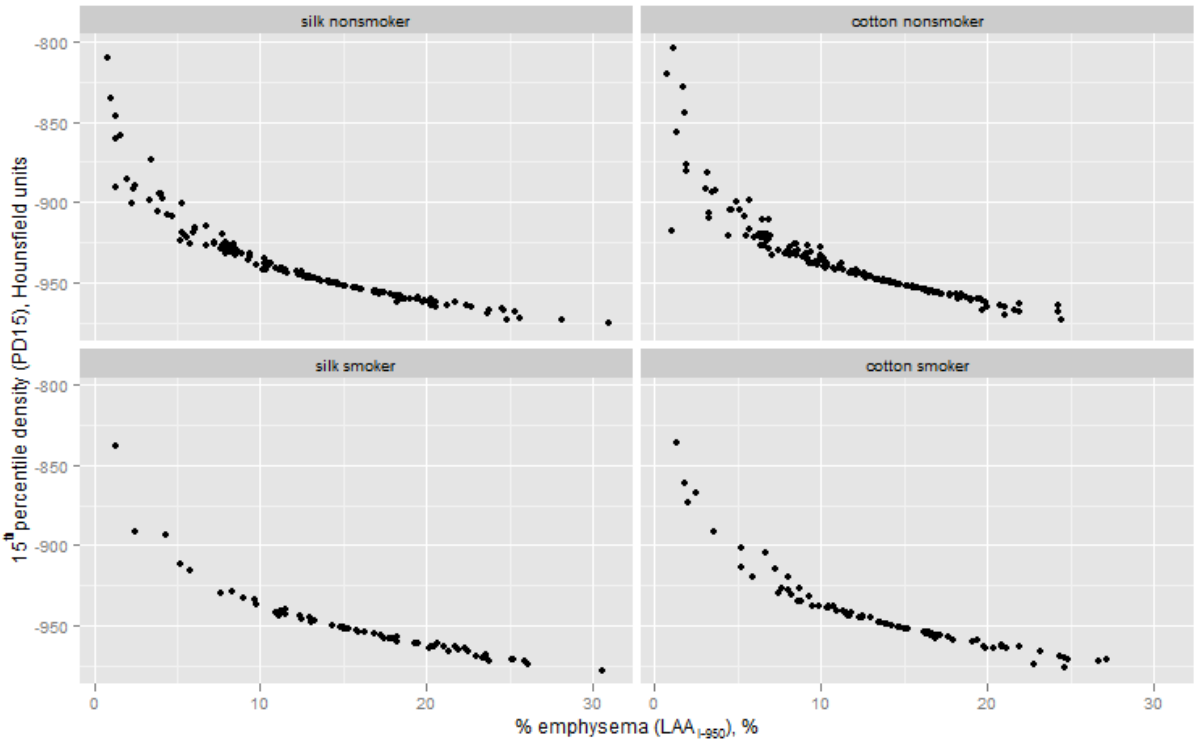

Study Start Date
Worker Retirement
Survey Years 1981 1986 1992 1996 2001 2006 2011
Subjects 919 787 787 684 688 635 587
Cotton 447 384 394 346 346 317 296
Silk 472 402 393 338 342 318 291
Cumulative Deaths 0 5 23 35 60 104 128
Lost to follow-up 0 127 109 200 171 180 204
Cumulative Data Collection Period
Spirometry
Modified ATS Symptom Questionnaire
Exposure Assessment
CT chest

Figure 2.


silk nonsmoker
cotton nonsmoker
silk smoker
cotton smoker
-800
-850
-900
-950
0
10
20
30
15th percentile density (PD15), Hounsfield units
% emphysema (LAA-950), %